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On the Effects of Constant Galvanic Currents upon the Mammalian Nerve-Muscle and Reflex Preparations.

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My experiments were to study the reflex effects resulting from stimulation by constant galvanic currents applied to an afferent nerve of the hind limb. The main points of enquiry have been: (1) the relation between reflex excitation and inhibition of the decerebrate tonus of the vasto-crureus; (2) whether the "excitation formula" (Pflüger's law) holds good in mammalian afferent nerves; and (3) whether there is continuous excitation during the passage of the current through an afferent nerve.

The reflex effects thus produced in the extensor muscle are complex and various, and are difficult to analyse without accurate knowledge of the results obtainable in the peripheral nerve-muscle and in the flexor reflex preparation. A few experiments were therefore made with these preparations, since the exact data required could not be obtained from the literature.

Method.—For the study of the reflex effects in an extensor muscle, the vasto-crureus of the decerebrate cat has been employed. The animal was decerebrated under deep anæsthesia with Sherrington's decerebrator. All the hind limb muscles except quadriceps were paralysed by denervation or tenotomy. The popliteal, peroneal or sciatic was used as the afferent nerve. The nerve was, though exposed previously, not actually isolated until everything was in readiness to observe the effects of stimulation, in order to avoid any changes resulting from exposure or injury to its vascular supply. The movements of the lower limb were recorded by fixing the condyle of the femur and attaching the tendo Achillis by means of a stout thread to one arm of a crank lever; the second arm, suitably weighted, functioning as a writing point. The ascent of the myogram line in the figures means contraction of the muscle; descent means inhibitory relaxation.

The constant current for stimulation was supplied usually from three accumulators. They were connected with both terminals of a rheochord, whose wire (of about 6 ohms resistance) was divided into 100 divisions. The numbers on the figures denote those of divisions of the wire short-circuited to stimulate the nerve. During the period marked by the downward notch in the signal line the constant galvanic current is applied to the nerve. Time below in seconds.

The distance between the non-polarisable electrodes upon which the nerve lay was usually 1·5 to 3 cm. and that from the ligated end to the distal electrode was more than 2 cm. The nerve was kept moist with warm saline or Ringer-Locke solution, or was sometimes enclosed in a glass tube, somewhat like the one described by Sherrington (11), in order to avoid evaporation. The direction of the constant current is described as "descending" (D, in the figures) when it flows in the afferent nerve from its cut end to the spinal cord, and as "ascending" (A, in the figures) when opposite in direction. This terminology is more reasonable in comparing the effects of constant current in the reflex and the peripheral nerve-muscle preparations, as the current in both cases passes through the nerve in the same direction with regard to the effector organ.

For the study of the reflex effects in a flexor muscle, the tibialis anticus of either the spinal or decerebrate cat has been used. The spinal transection was performed at the posterior end of the thoracic region. The nerve-muscle preparation was also tibialis anticus with its motor nerve (peroneal) isolated in the thigh. In these preparations, the distal end of the femur and tibia were fixed and the contraction of the muscle was directly recorded. Otherwise the method was similar to that already described for the extensor reflex preparation.

I.—EXPERIMENTS ON NERVE-MUSCLE PREPARATIONS.

The threshold value of stimulation for this preparation was very low as compared with that in reflex preparations. One or even half a division of the rheochord wire, through which a constant galvanic current flowed fed by one accumulator, usually suffices to provoke an appreciable contraction at closure of either descending or ascending current. With a fresh motor nerve the contraction at make generally appears with weaker ascending than descending currents. With currents of moderate strength in either direction excitation occurs both at make and break. This second stage of the "excitation formula" (Pflüger's law) appears with a rather weak current; two divisions of the rheochord are usually enough to induce opening contractions.

Very strong currents are, however, necessary to demonstrate the third stage of this formula. As a rule, I obtained no excitation at make of the ascending current, using the full potential difference obtainable from three accumulators (6 volts). It was, however, difficult to decide whether or not there was a contraction at make of the descending current, since the muscle was thrown into tetanus during its passage and appeared to relax without any further contraction when the flow of the current ceased (fig. 1). There is no doubt, however, that there is no contraction at break of a strong descending current in preparations where the motor nerve has been divided forty-eight hours before the experiment, as the muscle responds only at its make, remaining quiescent all the time the current is passing.

The height and form of the closure- and opening-contractions are different according to the strength and direction of the current. Though these contractions are not twitches in a strict sense, they are twitch-like if produced by weak currents. The contractions at make of the descending and at break of the ascending current increase with the intensity of the stimulation, while those at break of the former and at make of the latter attain their maximum, remaining on it within a considerable range, then diminish their height with further strengthening of stimulation, and finally fail to appear when the current is still stronger (fig. 1).

With weaker polarising currents there is no continuous excitation during their flow through the nerve in either direction. With progressive increase of the strength of the descending current, the make-contraction is no longer twitch-like, but is a brief tetanus, subsiding somewhat slowly. The stronger the current the more prolonged is the contraction, until such strength is reached as produces a contraction of the muscle, which lasts during the whole continuance of passage of the current. When the current is strong this closure tetanus is smooth and regular (fig. 1). During the flow of the current it declines at first quickly, then more gradually, and finally at break relaxes more or less quickly.

With the ascending current there is usually no continued tetanic contraction, whatever the strength of the current may be. A not infrequent exception to this rule is, however, that, with an ascending current of moderate strength, a weak and somewhat rapidly declining tetanus does occur during the passage of the current; but with a strong ascending current no contraction whatever occurs during the passage of the current, although on cessation of the stimulus a tetanus immediately ensues. This last seems to be a similar phenomenon to the "Ritter's opening tetanus" in the frog.

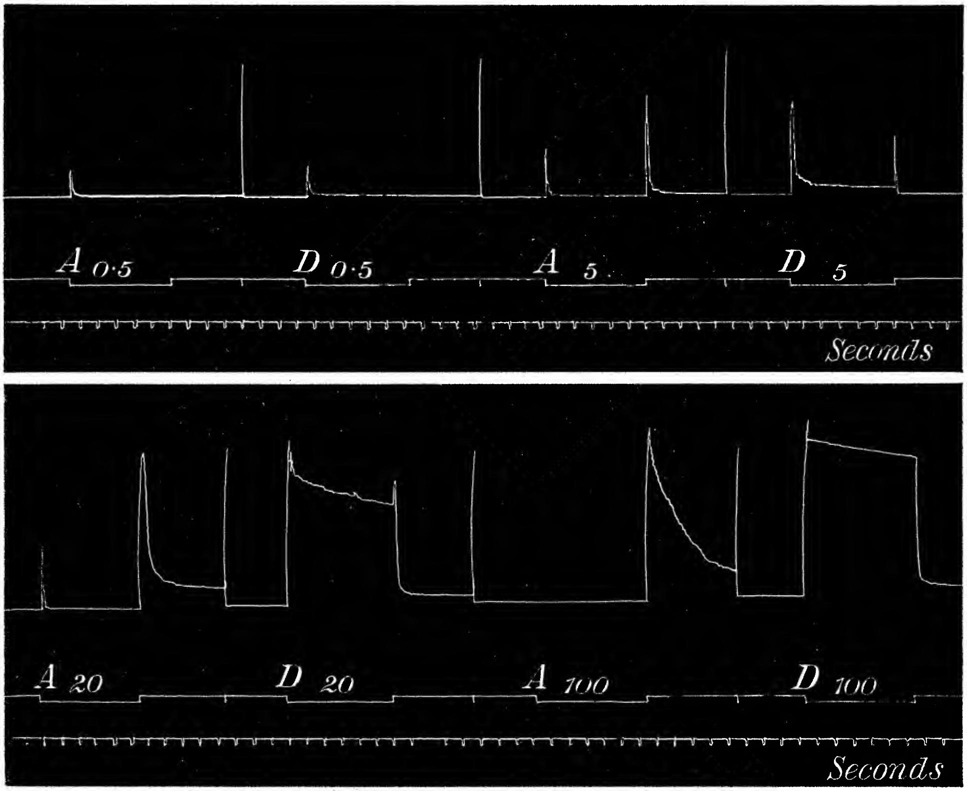

Fig. 1.—Nerve-muscle preparation of cat, showing response to constant current stimulation. A = ascending; D = descending.

The remarkable fact that continuous excitation is obtainable during the passage through a mammalian nerve of descending current of sufficient strength, while its muscle remains quiescent if the current is ascending in direction, was first observed by Eckhard (3). The difference which exists between the reactions given by the nerve-muscle preparations of cold-blooded and warm-blooded animals is partly explained by the extreme susceptibility of the isolated tissues of the latter to nutritional and temperature changes. Impairment due to these causes is progressive and depends on the period of exposure, as is shown by the following experimental observations:—(1) The threshold value of the effective stimulus increases and the height of the resultant contraction diminishes with lapse of time after isolation. (2) If the motor nerve has been exposed for some time, the break contraction is often produced with a smaller ascending current than is the make. (3) If the nerve is still more impaired, a constant ascending current causes an apparent relaxation of the muscle at make which is removed only at break (fig. 2). On the other hand, a small contraction occurs at make of the descending current, which is maintained during its flow. The damage to the nerve appears to be the cause of continuous stimulation, which induces without

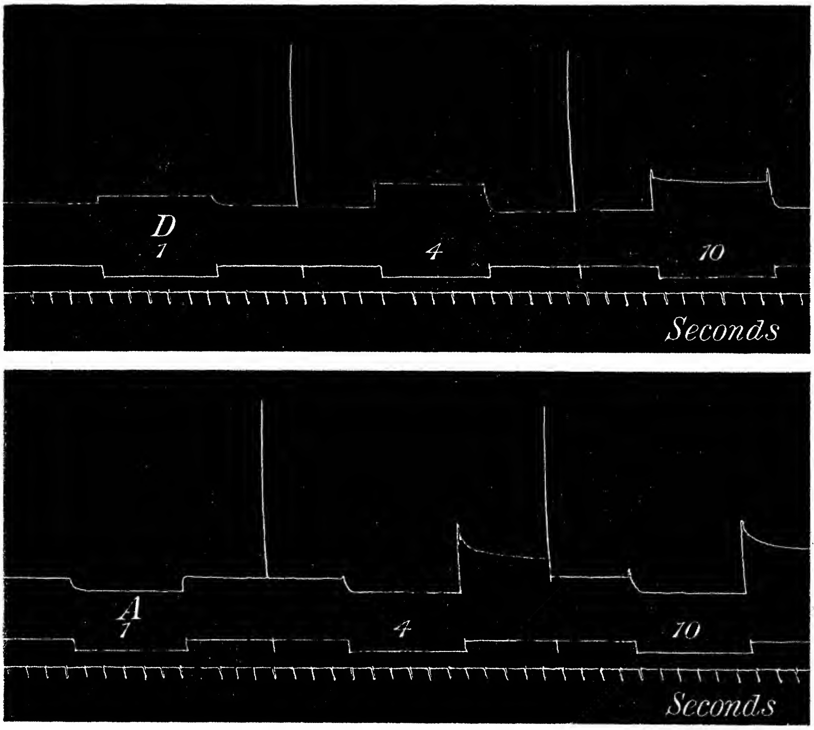

Fig. 2.—Nerve-muscle preparation of cat with the nerve left exposed after its isolation. The muscle is in a "tonic" contraction which is depressed by the ascending and augmented by the descending current.

any external stimulus, a weak tetanus, which is either augmented or depressed by the onset of catelectrotonus or anelectrotonus. It may be therefore supposed that in a fresh mammalian nerve, even immediately after isolation, subliminal stimuli due to injury are present, and when the impairment of the nerve is advanced, the muscle is already in a state of weak tetanus.

The closure and opening tetanus seen in the mammal may be explained in the same way as in the frog, if we assume that these subliminal stimuli due to injury and exposure become effective from the positive modification in excitability at the catelectrotonic tract of the nerve during the closure of the current, and at the previously anelectrotonic tract on opening the current.

The question arises here as to whether these pre-existing stimuli are due to an injury current or to other intrinsic causes connected with the dying process of an isolated tract of the nerve. To decide this, the sciatic nerve was divided twenty-four or more hours before the experiment, since the injury current diminishes greatly in that time. The results obtained in such preparations were identical with those already described. The injury current does not appear, therefore, to play an important *rôle* in this respect. When the animal is left alive more than forty-eight hours after aseptic division of the sciatic nerve, the threshold value of the nerve rises, and continuous excitation is no longer obtained during the passage of strong currents in either direction. Even in the third stage of the excitation formula, no appreciable tetanus takes place during the flow of descending current through the nerve. The nerve loses entirely its mechanical and electrical excitability if the animal is allowed to survive three days after the nerve section. This reaction of degeneration does not directly concern us here.

II. EXPERIMENTS ON THE FLEXOR REFLEX PREPARATION.

These results are on the whole similar to those obtained in the nerve-muscle preparation. The threshold value for the spinal preparation was often found to be nearly as low as that of the nerve-muscle preparation. It was, however, much higher in the decerebrate (sometimes more than ten times higher) than in the nerve-muscle preparation (Sherrington (14) and others).

Pflüger (7) himself showed that the law which he formulated for the motor nerves held for the afferent nerves of the frog. In the mammalian reflex preparation, the second and third stages are more easily demonstrated than the first. The first stage can usually be verified if the threshold value is low. When it is high, as in the decerebrate preparation, the contraction at break of the ascending current often makes its appearance at a lower value of the current stimulus than does the contraction at make, while the reverse is true of descending currents.

In some flexor preparations, both decerebrate and spinal, with freshly isolated nerves, no continuous excitation takes place during the passage of either fairly strong ascending or descending currents. More usually, however, reflex tetanus is produced at make of the current, if the current be strong and of descending direction. The intensity of current necessary to produce this result is far greater in these preparations than in the nerve-muscle preparation. Moreover, this closure reflex tetanus usually subsides some time during the course of prolonged stimulation.

With the flexor reflex preparation therefore, even when that preparation is of the spinal kind, which is more readily responsive reflexly than the decerebrate, the galvanic current even when strong evokes from the afferent nerve a contraction only at make and break, and not during the maintained passage of the current, or does so only exceptionally. This is a striking difference from the nerve-muscle preparation.

III.—EXPERIMENTS ON THE EXTENSOR REFLEX PREPARATION.

The experimental results in this preparation are more complex, and it is sometimes difficult to analyse the various factors concerned in causing this complexity. Under favourable conditions the results, though manifested in a different way, agree in regard to the effects of changes in strength and direction of current with those obtained in the nerve-muscle and flexor reflex preparations. These reactions can be more easily understood when the influence of certain characteristic features of the central nervous system is considered. Weaker or less abrupt stimulation of an ipsilateral nerve, as Sherrington and Sowton (12) showed, tends to produce contraction in the knee-extensor, while stronger or more abrupt stimulation always results in inhibitory relaxation. Constant currents are more suitable for the study of this phenomenon than induction shocks. The other important features of the central nervous mechanism which are related to the present enquiry are tonic plasticity (10) and the "rebound" phenomenon.

The general experimental results with this preparation are recorded in four sections.

(1) *The Validity oj Pflüger's Law.*

In excitable preparations, the reflex movement, whether excitatory or inhibitory, appears with smaller intensity of stimulation at make of the either descending or ascending current than at its break. If, however, the threshold value is rather high from the first the reflex effect at break of the ascending current has a threshold which is either lower than or equal to that at its make (see above). Even in this case it is always greater at make of the descending current than at its break. This result agrees with that obtained in the flexor reflex preparation. It corresponds to a stage of Pflüger's law which in the nerve-muscle preparation is reached only after the current has been made stronger than that which gives the initial stage of the law. The

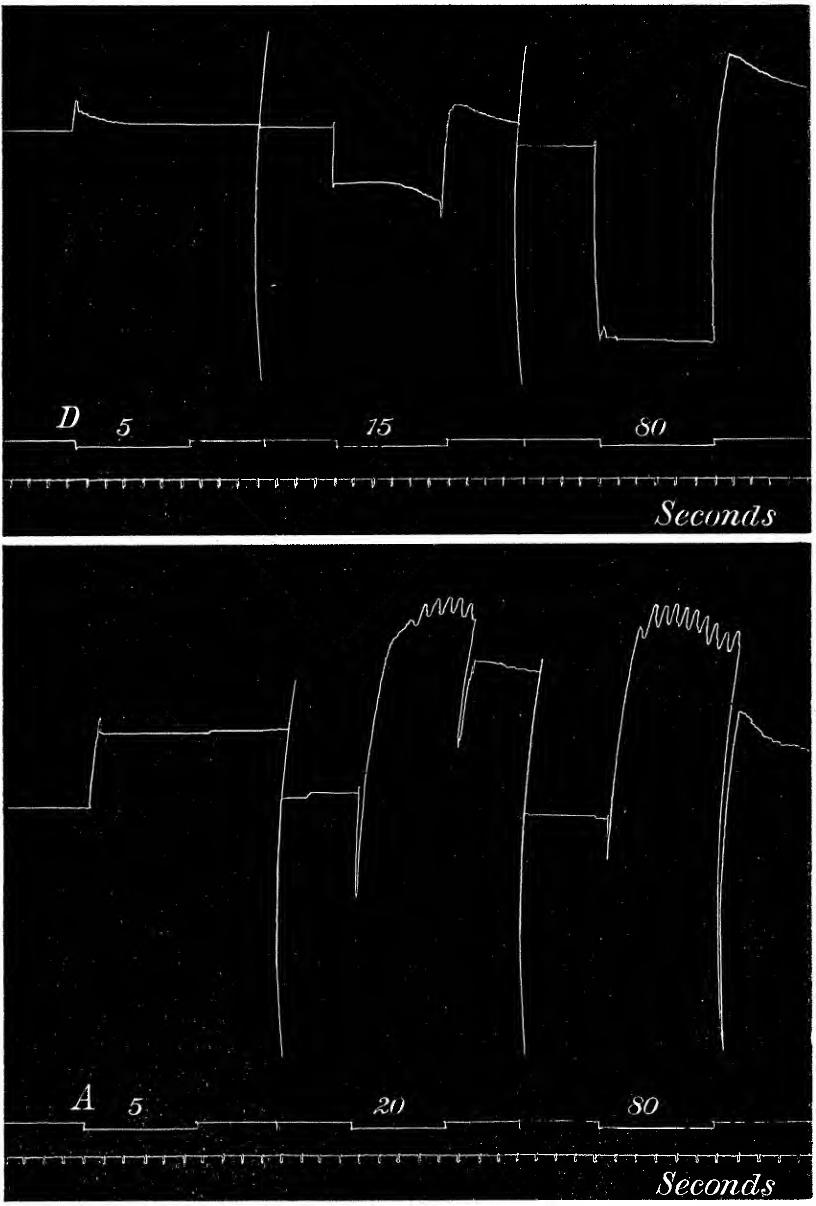

Fig. 3.—Extensor reflex preparation of decerebrate cat, showing response to constant current stimulation, applied to the proximal stump of the cut sciatic nerve.

second stage of the law of polar excitation is attained by doubling the intensity of the current and is still elicited within a considerable range. By further intensifying the stimulation, the reflex effect produced by closing the descending, and by opening the ascending current, increases progressively to a maximum (fig. 3).

It is often difficult to make out the relation between the intensity of the current and the extent of the reflex effects produced at break of the descending, and also, though in a less degree, at make of the ascending current, since many factors are involved in the reaction. Under favourable conditions of experiment, as in the preparation where any reflex effect is only inhibitory, these reflex effects sooner or later attain a maximum at a certain optimal strength of current, and then gradually diminish in extent with further increase of strength of the current, so that the third stage of Pflüger's law holds good in the extensor reflex preparations.

(2) *The Relation between Reflex Excitation and Inhibition.*

In preparations of good decerebrate rigidity, excitation is usually produced by a weak current, both at make and at break, in either direction. The myogram line rises abruptly and either drops down again more or less quickly to the initial line or remains at the new position. When this shortening reaction following an active reflex contraction is well pronounced, and the stimuli at both make and break take effect, the myogram exhibits two steps. The excitatory reflex effect increases with the strength of current within somewhat narrow limits. As the current is increased, the reflex contractive effects produced at make of the descending and at break of the ascending current, first of all diminish in extent, and finally pass over into inhibitory relaxation which becomes progressively greater with further strengthening of the stimulus.

Similarly, at make of the ascending and at break of the descending current reflex contraction gradually changes into inhibitory relaxation when the strength of the current is step by step increased. The relaxation in this case is always limited. In some preparations where the decerebrate rigidity is well marked, no relaxation of the muscle is induced at all, and only excitatory effects are obtained, whatever the strength of the current employed. This result may be explained by assuming that, as the stimuli at make of the ascending and at break of the descending current do not exceed a certain intensity, such relatively weak stimuli in such preparations cause only excitation.

(3) *On Continuous Excitation in the Nerve during the Passage of the Current.*

During the flow of weak constant galvanic currents in either direction, no continuous reflex effect occurs. With stronger stimulation, the reflex phenomena obtainable during the passage of current through the nerve are different according to its direction, as was seen by Sherrington and Sowton (12). With the ascending current, the excitatory effect at its make increases progressively with the strength of excitation and then often passes over into a rebound contraction following an inhibitory relaxation of short duration. The rebound contraction is, in its turn, followed by a shortening reaction or relaxes gradually. A passive plastic reaction is easily obtained during the passage of strong ascending currents, except immediately after its closure when the rebound phenomenon is still in progress. This is a convincing proof that no excitation is occurring during the passage of even a strong ascending current. This rebound phenomenon is, however, often so prolonged that one may think that excitation is still in progress. In such a case, the lengthening reaction is more limited than before the application of the current. This excitatory process is neither more nor less than a prolonged rebound phenomenon. If the preparation is of poor rigidity, where any reflex effect is only inhibitory relaxation of the tone of the muscle, the effect at make of a constant ascending current of sufficient strength is inhibition followed by a lengthening reaction without any rebound contraction. This is another sign that there is no continuous reflex effect during the passage of the current, since the plastic reaction is unchanged by its application.

At make of a stronger descending current the tonic contraction of the muscle relaxes. This relaxation is somewhat prolonged, and is followed by a rebound contraction weaker than that produced by a like stimulus of ascending direction. This seems to correspond with the result seen in the nerve-muscle preparation, that the contraction at make of currents of moderate strength is prolonged in its phase of relaxation. The poor rebound phenomenon may result from depression by a prolongation of the inhibitory effect at make, so that the resulting level of the myogram line is determined by the algebraic sum of excitatory and inhibitory processes. By further increase in the intensity of stimulation, the inhibitory relaxation becomes more complete and the succeeding rebound contraction is less marked, until the former attains its full extent and the latter no longer takes place. The phenomenon is not merely a lengthening reaction following inhibition, since the muscle is felt to be more flaccid than before the application of the current, and the passive shortening reaction is no longer easily obtainable. If the lower limb is then passively extended, so that the myogram line is at a high level, this falls slowly and steadily during the flow of a strong descending current. The strength of stimulation required to produce a continuous effect in this case is far greater than that in the case of nerve-muscle preparations. The inhibitory reflex effect at break of a powerful ascending current is often prolonged. The rebound contraction following it, is limited, as it is depressed by prolongation of the inhibitory process.

The above experimental data show that there is a continuous reflex effect during the passage of a strong descending polarising current through the afferent nerve in the extensor reflex preparation, which is not present if the current is reversed in direction. The results in this preparation of stimulation by constant galvanic currents show full agreement with those in the flexor reflex and nerve-muscle preparations. In order to produce a continuous effect from the afferent nerve, the current required is, however, far stronger than that for the nerve-muscle preparation.

These reflex results in the extensor reflex preparation bear some resemblance to the changes in the respiratory movement observed by Langendorff and Oldag (5), as a result of the application of constant galvanic currents to the central stump of the cut vagus.

In some preparations in which walking movements occurred without any artificial stimulation, these movements were inhibited whenever a weak polarising current was led into the nerve in descending direction. On the contrary, ascending currents either augmented the movements or produced no effect upon them. Sometimes, in the latter case, the walking movements, which were not present before stimulation, were superadded to the usual reflex results (fig. 3).

(4) *The Response by Excitation or Inhibition modified by Factors other than those involved in Stimulation.*

The complexity and multiplicity of reflex results in the extensor reflex preparations are dependent on other factors than those already mentioned as influencing the functional condition of the central nervous mechanism. In the presentation of the results of these experiments, as detailed above, the more typical have been selected. There were, however, cases in which the responses were altered, by variation in the degree of decerebrate rigidity, in the initial length of the tonic muscle, and in the position of the neck and other joints:—

(i) The intensity of decerebrate rigidity developed in the preparation varies in different cats and is influenced by the depth of anæsthesia and the time which elapses after decerebration. In some preparations, especially those with poor rigidity, excitatory reflex effects are entirely, or almost entirely, absent, and only inhibitory reflex effects can be obtained. No rebound phenomenon occurs even after strong stimulation. Plastic reaction is, however, fairly easily obtainable. At a certain stage of anæsthesia, a preparation showing typical reflex responses of excitation and inhibition before administration of the drug is converted to one in which the excitatory reflex effect is entirely excluded. The anæsthetic (chloroform and ether) can convert an excitatory into an inhibitory reflex effect, as was first pointed out by Bayliss (1) in the case of the vasomotor pressor reflex, and by Sherrington and Sowton (13) in the vasto-crureus reflex preparation. In other preparations, on the contrary, excitatory effects are obtained with comparatively strong currents in either direction. Only the strongest stimulation of the afferent nerve produces relaxation of the tonic contraction of the muscle, and the inhibition is always followed by a marked rebound contraction.

(ii) Another condition which influences the excitatory and inhibitory effects is the initial length of the muscle. The muscle in the lengthened condition reacts by excitation more easily to any stimulation, and in the shortened condition by relaxation. If the functional condition of the tonic centre favours the extensor tone, the reflex effect as obtained by weak stimulation of the afferent nerve is often excitation, whether the muscle be lengthened or shortened. The contraction in the former condition is always more ample than that in the latter. Conversely, if the condition of the tonic centre is unfavourable to the tone of the extensor muscle, the reflex effect is usually relaxation, irrespective of the length of the muscle, especially with strong stimuli. The inhibitory relaxation is always greater in this case, the more shortened the muscle. Between these two extremes it is often observed that reflex contraction is induced by a given stimulus when the muscle is lengthened, while the inhibition takes place with the same stimulus when it is shortened (fig. 4).

It has been noticed that the posture of the limb influences and can even reverse the direction of reflexes obtainable from the limb (6, 8, 9). But such observations have rested on experiments in which there has been no actual isolation of the muscle which responds to the reflex stimulus employed. In the present instance the observations I have obtained have been made on the actually isolated muscle.

(iii) The position of most of the other joints in the body has some influence upon the mode of reflex reaction (Beritoff (2), Magnus (6)). Above all, the position of the neck in regard to the trunk and that of the head in space must be kept constant throughout the experiment when the relation between excitatory and inhibitory reflex effects is under investigation. Reflex reversal is often obtained by twisting the neck from one side to the other (fig. 5).

The reflex reversal by altering other conditions of experiment than those here described, does not, however, directly concern the present experiments.

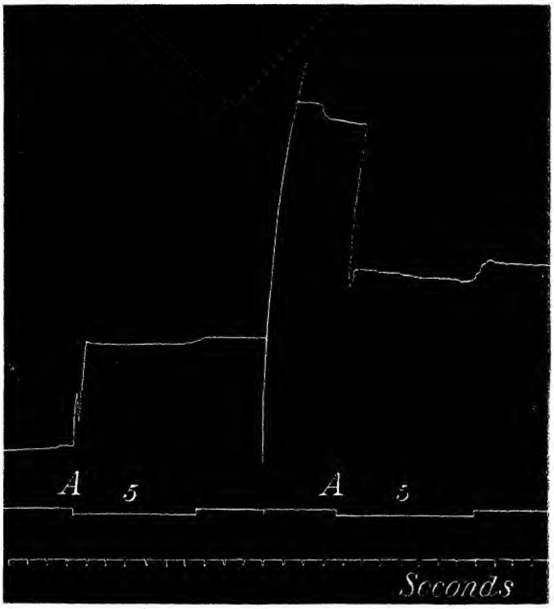

Fig. 4.—Extensor reflex preparation of decerebrate cat, showing reflex reversal by alteration of initial length of the tonic muscle.

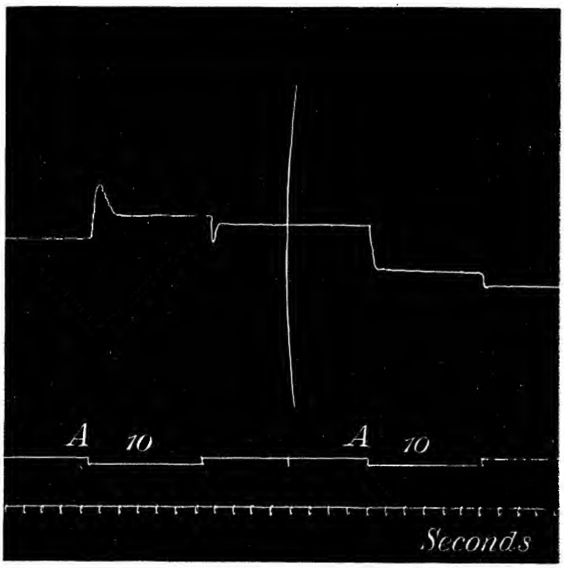

Fig. 5.—Right extensor reflex preparation of decerebrate cat, showing reflex reversa obtained by altering the posture of the head from the right to the left side.

In regard to those other conditions, the article, "Reflex-umkehr" ('Ergebnisse der Physiologie') by Graham Brown (4), where the cases are exhaustively summed up, may be consulted.

Conclusions.

(1) The "excitation formula" (Pflüger's law) holds good in mammalian nerve both efferent and afferent. When the threshold value of stimulation is high, as in some decerebrate reflex preparations, the contraction at break of the ascending current takes place in an earlier stage than that at its make, though the reverse is true if the current is descending.

(2) During the passage of a weak constant galvanic current, no continuous excitation is produced in either nerve muscle or reflex preparation. If a stronger descending current is passed through the nerve, this occurs in both preparations; though if the current is ascending, this is usually not the case. Far stronger currents are necessary to produce it in the reflex than in the nerve-muscle preparation.

Two days after division of the sciatic nerve, no continuous excitation is produced during the passage through the nerve of constant current, however strong, either ascending or descending.

(3) In nerve-muscle preparations, if the nerve is in bad condition after prolonged exposure, it acquires the character of responding more easily, with tetanus at the make of the descending and at break of the ascending current. When the condition is advanced the muscle seems to be in a weak contraction even when no stimulus by the current is being given to it: the passage through it of an ascending current then causes a relaxation of the muscle during the passing of the current.

(4) As to the relation between reflex excitation and reflex inhibition the following can be stated:—

The intensity of the inhibitory and excitatory reflex effects on the tonus of the vasto-crureus muscle and the relation between these two effects are determined by the strength, duration and direction of the stimulating current, but are also influenced by the degree of tonus in the preparation, the initial length of the muscle, and the position of the neck and other joints. Any condition which favours the tonus of the extensor muscle causes increase in the excitatory tendency of the reflex effect and diminution in the inhibitory tendency, and *vice versâ*. The reversal of reflex effect is thus often obtained by altering these conditions without changing those of stimulation.

I am greatly indebted to Prof. C. S. Sherrington for suggestions and advice during the course of these experiments.

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On the Longevity of certain Species of Yeast.

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In the autumn of 1918 Dr. J. J. Hood handed to one of us (A. R. L.) eight cultures of yeast, belonging to the late Mr. A. Gordon Salamon. The cultures in question, it appears, were given to Mr. Salamon by the late Prof. Hansen in 1887. They consisted of Freudenreich flasks, containing wads of perfectly dry cotton-wool. The flasks were furnished with a side tube and there was a tube at the apex of each hollow stopper. There was a cotton-wool plug at the opening of each flask inside the hollow stopper, and the tube at the apex of the stopper was plugged with cotton-wool, as was also the side tube. In addition to this, the cotton-wool plug of the side tube was coated with sealing-wax and the hollow stopper was also fixed on with a ring of sealing-wax. Each flask bore a label, giving the name of the particular yeast and